

**CHEMICAL AND PHYSICAL CHARACTERISTICS OF MAHONING
RIVER SEDIMENT BEFORE AND AFTER FUNGAL BIOREMEDIATION**

By

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Submitted in Partial Fulfillment of the Requirements

for the degree of

Master of Science

in the

Chemistry

Program

**YOUNGSTOWN STATE UNIVERSITY
MAY 2008**

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ABSTRACT

The Chemical and Physical properties of Mahoning River sediments were investigated in this study. Experiments were conducted to determine how fungal remediation affected availability of toxic metals in contaminated Mahoning River sediment. Samples were analyzed before and after bioremediation. Sediments were treated with sawdust and sawdust plus *Pleurotus ostreatus*. The total concentrations of arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), selenium (Se) and silver (Ag) were determined by microwave assisted total acid digestion. Availability of toxic metals was assessed by toxicity characteristic leaching procedure (TCLP). Metals were analyzed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). The concentrations of most metals were above ecological screening levels as confirmed by total metals study. Most of the TCLP metals detected were below the USEPA regulatory limit. Sediment treated with fungi showed a 15 – 40 % reduction in soil concentrations of As, Ag, Ba, Cd, Cr, and Pb. More controlled study with many samples is suggested to determine the trend accurately. There was no effect of these treatments on physical properties such as moisture content, ash value and particle size distribution.

ACKNOWLEDGEMENTS

I wish to thank my advisor, Dr. Carl G. Johnston for his help, encouragement, apposite guidance, and time throughout this project. You were there at every step, at any time for the support. I also thank you for providing me an opportunity to work with you and providing all the resources for my work. This project would not have been possible without you.

My special thanks to Dr. Daryl Mincey, Chair of Chemistry Department at Youngstown State University (YSU), for encouraging me to work on this project, for helping me with all necessary departmental and logistic support and for making me familiar with Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES). I would also like to thank Dr. Josef Simeonsson, Associate Professor, Chemistry Department at Youngstown State University for guiding me with the usage of Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES) and analytical procedure and calculations. My thanks to our research group members Ms. Sowmya Pabba, Ms. Sarala Sajja, Mr. Gregory Bosilgcic and Mr. Matthew Gacura. Without a team effort the project wouldn't have been possible. I would like to thank the School of Graduate Studies and Research at YSU for providing me a research assistantship (RA) and STEM for providing funds for analyses during the study. I like to thank Dr. Tim Wagner for his advisement and support. I also thank our department secretary Ms. Kris Gregory for all her help. I would like to thank Mr. Ramana Konda and Mr. Looja Tuladhar for their company during my study at Youngstown State University.

Finally, mom and dad, it's your, support, encouragement and blessing that has been very crucial behind whatever I have achieved so far, thank you.

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF FIGURES	vi
LIST OF TABLES	vii
 CHAPTER	
1.0 INTRODUCTION	
1.1 Background	
1.1.1 Mahoning River.....	1
1.1.2 Goals of the study.....	2
1.1.3 Project Action Plan.....	2
1.2 Iron and steel industries and metal emission.....	2
1.3 Iron and Steel industries in Mahoning valley and air/river pollution.....	3
1.4 Heavy metals in soil sediment and their sources.....	7
1.5 Hazardous metals their sources and health impact.....	8
1.6 Heavy metals bioremediation in soil sediment.....	10
 2.0 INSTRUMENTATION, METHODS AND PROCEDURES	
2.1 Inductively Coupled Plasma- Atomic Emission Spectroscopy.....	13
2.2 X-Ray Fluorescence spectroscopy.....	15
2.3 Toxicity Characteristic Leaching procedure.....	15
2.3.1 Overview.....	15
2.3.2 Scope and Application.....	16
2.4 Microwave Assisted Acid Digestion.....	18
2.4.1 Overview.....	18
2.4.2 Scope and Application.....	18
2.5 Moisture and Dry Matter content.....	18
2.6 Ash Value.....	19
2.7 Particle Size Analysis.....	19
2.8 Bio-treatment and Incubation.....	21
 3.0 RESULTS AND DISCUSSION	
3.1 Metal content in TCLP extract using ICP-AES analysis.....	22
3.2 Overall TCLP discussion.....	24
3.3 Metal content in Microwave assisted total acid digestion samples using ICP-AES.....	25
3.4 Overall TAD discussion.....	28
3.5 Metal content using XRF.....	29

3.6	Moisture content.....	30
3.7	Ash content.....	31
3.8	Particle size analysis.....	32
4.0	SUMMARY, CONCLUSION AND RECOMMENDATION	
4.1	Summary and Conclusion.....	34
4.2	Recommendation.....	35
	REFERENCES.....	36
	APPENDIX 1	
1.1.1	Procedures for Chemical Properties.....	41
1.1.2	Toxicity Characteristic Leaching Procedure.....	41
1.1.3	Microwave Assisted Acid Digestion.....	42
	APPENDIX 2	
2.1	Procedures for Physical Properties.....	44
2.1.1	Moisture Content/Dry Matter.....	44
2.1.2	Ash Value.....	44
2.1.3	Particle Size Analysis.....	46
	APPENDIX 3	
3.1	Bio-treatment and Incubation.....	48
	APPENDIX 4	
4.1	Inductively Coupled Plasma- Atomic Emission Spectroscopy.....	50
	APPENDIX 5	
5.1	X-Ray Fluorescence Spectroscopy.....	55

LIST OF FIGURES

Figures	Page
1-1. Air Emissions from Steel Industries in Mahoning Valley.....	5
3-1. Toxicity characteristics leaching procedures for Barium.....	22
3-2. TCLP for Chromium.....	23
3-3. TCLP for Lead.....	24
3-4. Total acid digestion of Silver.....	25
3-5. Total acid digestion of Arsenic.....	26
3-6. Total acid digestion of Barium.....	26
3-7. Total acid digestion of Cadmium.....	27
3-8. Total acid digestion of Chromium.....	28
3-9. Total acid digestion of Lead.....	28
3-10. % Moisture content.....	31
3-11. % Ash weight.....	32
3-12. Sediment composition in triplicate run.....	33
A3-1. Mixing protocol for samples on day 21 with and without nitrogen source...	49
A4-1. ICP Torch.....	52
A4-2. Atom formation in ICP nebulizer.....	53
A5-1. XRF Process.....	56
A5-2. Quantitative X-Ray fluorescence scans.....	57

LIST OF TABLES

Table		Page
1-1.	Inventory of release of pollutants from iron and steel facilities.....	6
2-1.	Wavelengths (nm), method detection limit and reporting limit for ICP analysis of elements.....	14
2-2.	TCLP regulatory limits for some hazardous materials.....	17
2-3.	Elements those are applicable for microwave digestion.....	18
2-4.	Hydrometer and temperature readings for Particle size analysis.....	20
2-5.	Sample bio-treatment protocol.....	21
5-1.	XRF analytical lines for some elements.....	58

CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Mahoning River

The Mahoning River is considered one of the most polluted rivers in the United States. There are still both point and non-point sources of pollution in the Mahoning river valley, however according to the US Environmental Protection Agency Region V report, enormous loading of the contaminants occurred during the previous industrial era as recently as 1977 from nine major steel plants. Major contaminants comprised of suspended solids, grease, ammonia-nitrogen, cyanide, phenolics, heavy metals and oils. The deposition of these pollutant residues over time resulted in the degradation of the aquatic ecosystem and has become a threat to public health. According to Schroeder (Mahoning River Corridor redevelopment Project, 1994), the Mahoning River water contained high concentrations of metals such as copper, zinc, lead, chromium, iron, nickel and cadmium along with other pollutants. The Ohio Department of Health (DOH) has recognized a possible impact to human health for at least two decades. In 1988, the agency issued a human health advisory against contact with the sediment on the river bottom and along the banks. Consumption of fish from the area was discouraged. The principal problem identified in various studies (OEPA Report, 2006 & 1996) in the Mahoning River area is the degraded condition of the river ecosystem which leads to the depressed values for standard indices of biological health (OEPA Report, 2003 & 2007). The benefit associated with the cleaning and restoration of Mahoning River outweighs the cost associated in this process (www.ysu.edu/mahoning_river). The principal

opportunity is to return the river and its ecosystem to a natural environment with healthy aquatic communities (Phase II treatability).

1.1.2 Goals of the study

1. Study the physical and chemical properties of river sediment
2. Determine the impact of fungal bioremediation on these properties.
3. Determine the impact of fungal bioremediation on availability of toxic metals.

1.1.3 Project action plan

The action plan for the study included collection of river sediment from Lowellville, OH. The sediments were treated with sawdust, paper, fungi and a source of fungal nitrogen and then incubated at 25°C. Samples were taken for chemical (metals) and physical analyses on day 0 and day 42 of treatment. Total concentration of several heavy metals (by microwave assisted total acid digestion method) and available metals by toxicity characteristic leaching procedures (TCLP) were analyzed. Physical properties measured were moisture content, dry matter content, ash weight, and particle size analysis on day 0, day 21 and day 42.

1.2 Iron and steel industries and metal emission

Iron and steel are used in the production of a wide range of products, including food storage containers, defense applications, and ship hulls. The two basic processes of steel manufacturing include,

- Use of a blast furnace by “integrated” steel mills to produce iron from iron ore, coke, and fluxing agents. A basic oxygen furnace is then used to convert the molten iron, along with up to 30% steel scrap, into refined steel.

- Use of an electric arc furnace by “minimills” to melt steel scrap and limited amounts of other iron-bearing materials to produce new steel. The scrap metal used in steel production originates from sources such as scrapped automobiles, demolished buildings, discarded home appliances, and manufacturing returns.

The integrated iron and steel industry encompasses all the steps included in the manufacture of steel from iron ore and other materials. Major processes in the production of finished steel include coke production, sinter production, iron making, steelmaking, alloying, casting and shaping, and finishing. Finishing processes, such as rolling mills, are similar at both types of mills (USEPA, 1995 & US Department of Energy, 2000).

The basic oxygen furnace, sintering plant, electric arc furnace, and hydrochloric pickling lines were considered to have the greatest potential to emit hazardous air pollutant substances (HAPs) including heavy metals (Marsosudiro, 2002). Most of the HAPs generated in the blast furnace are heavy metals, including cadmium, chromium, lead, manganese, and nickel. Heavy metal HAPs may be released (as particulate) from the iron ore and from the sinter operation. Also, as in the case for the blast furnace, most of the HAPs generated in the basic oxygen process furnace (BOF) are heavy metals, including cadmium, chromium, lead, manganese, and nickel (Marsosudiro,2002).

1.3 Iron and Steel industries in Mahoning valley and Air/River pollution

Youngstown is about 65 miles from Lake Erie and about 35 miles from the Ohio River. Since the district had surface iron ore, limestone, and timber from which charcoal could be produced, it led to the construction of the first blast furnace in the Mahoning

Valley District. It was established around 1803 on Yellow Creek and was called "Hopewell Furnace". The iron industry grew rapidly from 1845 to 1875. No less than 21 blast furnaces, with a combined capacity of approximately 250,000 tons per year were established in this district. There were 25 furnaces by 1920 and the production capacity had increased to 4 million tons per year. At its peak, the Youngstown-Pittsburgh steel district alone produced about 11 percent of the country's total (www.youngstownsteel.com). The industry started to grow again from the early 1940's and continued until the early 1970's. The Mahoning Valley alone produced about 30% of the Ohio State's total through those years. The iron and steel industry in the area started to decline around 1970 and never substantially recovered.

Air pollution is one of the most visible environmental impacts of the steel industry. Particulate air pollution is a mixture of solids and liquid droplets suspended in air comprised of many different compounds, including acids, organic chemicals, metals, soil or dust (Godish, 2004). Other main sources of particle pollutants include power plant, automobiles, industrial plants and forest fires.

Although historical data on air pollution in Youngstown is not available, longtime residents recall brown and smoky skies, colored river water, dead fish, and health problems. 'Black rain' incidents in the mid 1940's indicate the alarming nature of air pollution in the Mahoning Valley. The steel industry, undoubtedly, was to blame for most of this pollution. Figure 1-1 shows the air emissions of some of the steel industries in the Mahoning Valley.

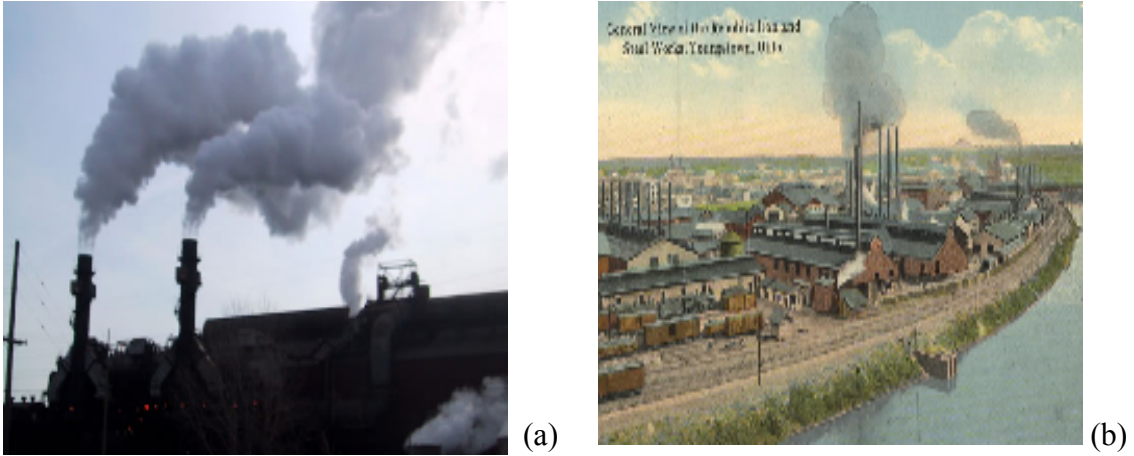


Figure 1-1. Air Emissions from Steel Industries in Mahoning Valley.

(a) Youngstown Sheet and Tube Company, Youngstown, OH

(b) Republic iron and steel works, Youngstown, OH

Source: www.familyoldphotos.com

It is difficult to find the exact quantity of heavy metals disposed by each industrial plant. An example of emission data, based on a survey of a large number of iron and steel industries, is presented in Table 1-1.

Table 1-1. Inventory of Release of Pollutants from Iron and Steel Facilities.

Source: EPA Sector notebook project: Profile of the iron and steel industry, (EPA/310-R-95-005)

(1993 Releases reported in pounds/year)								
CHEMICAL NAME	# REPORTING CHEMICAL	FUGITIVE AIR	POINT AIR	WATER DISCHARGES	UNDERGROUND INJECTION	LAND DISPOSAL	TOTAL RELEASES	AVG RELEASE PER FACILITY
SULFURIC ACID	157	385,882	321,639	27,700	0	4,705	739,926	4,713
MANGANESE COMPOUNDS	110	472,855	808,182	145,585	4,800	21,251,405	22,683,837	206,217
CHROMIUM COMPOUNDS	108	19,821	87,971	53,107	4,800	1,953,629	2,119,328	19,623
ZINC COMPOUNDS	108	594,037	874,383	121,804	250	13,497,412	15,090,083	139,723
HYDROCHLORIC ACID	102	612,814	1,468,636	25	11,726,300	744	13,309,519	135,367
CHROMIUM	95	10,858	24,826	4,432	0	415,839	456,055	4,301
MANGANESE	94	38,655	42,782	79,069	0	791,189	951,695	10,124
NICKEL COMPOUNDS	86	9,030	12,107	11,007	1,100	654,514	687,758	7,997
NICKEL	83	10,505	18,817	9,480	3,200	126,359	169,371	1,041
NITRIC ACID	86	86,647	487,887	39	0	44,730	629,303	9,535
LEAD	61	34,634	107,468	17,088	0	126,479	285,669	4,683
LEAD COMPOUNDS	61	55,583	76,024	11,559	0	1,087,501	1,230,677	20,175
AMMONIA	59	5,162,886	1,012,664	4,836,185	360,000	6,479	11,378,214	201,326
PHOSPHORIC ACID	56	73,666	7,672	280	0	142,814	229,412	4,097
COPPER COMPOUNDS	51	10,474	81,731	8,918	1,100	1,518,033	1,620,256	31,770
COPPER	36	17,281	4,902	3,237	0	16,320	41,740	1,159
ZINC (FUME OR DUST)	36	328,088	322,973	58,831	0	3,571,000	4,280,895	118,914
XYLENE (MIXED ISOMERS)	32	172,712	76,091	510	0	274	249,587	7,800
HYDROGEN FLUORIDE	30	96,276	133,328	19	0	20,789	250,412	6,347
TOLUENE	30	222,938	408,307	513	0	328	632,286	21,076
NAPHTHALENE	26	93,880	35,809	1,830	15,000	300	151,829	5,840
BENZENE	24	482,755	347,643	911	7,000	600	338,908	34,955
CYANIDE COMPOUNDS	24	14,828	91,828	72,033	41,000	909	220,798	9,200
CHLORINE	23	16,510	6,409	48,910	0	0	71,829	3,123
ETHYLENE GLYCOL	21	52,505	255	99,306	0	6,950	159,016	7,572
ETHYLENE	20	194,170	771,732	0	0	0	967,902	48,395
BARIUM COMPOUNDS	19	847	1,260	12,523	0	140,857	155,487	8,184
1,1,1-TRICHLOROETHANE	19	1,184,783	160,842	0	0	0	1,345,735	70,828
ANTHRACENE	17	3,830	11,636	9	0	0	15,475	910
PHENOL	16	101,803	77,677	30,445	76,000	23,817	309,842	19,365
ALUMINUM (FUME OR DUST)	15	5,536	56,373	22,522	0	210,064	294,697	19,646
PROPYLENE	15	23,148	81,649	0	0	0	109,798	7,320
METHANOL	14	487,708	18	0	0	35	487,762	34,840
DIBENZOFURAN	13	2,571	29	0	0	0	2,600	200
MOLYBDENUM TRIOXIDE	13	823	852	1,880	0	6,430	10,085	776
ETHYLBENZENE	12	13,504	3,803	250	0	0	17,557	1,463
TRICHLOROETHYLENE	12	572,277	484,600	5	0	0	1,056,882	88,074
AMMONIUM SULFATE (SOLUTION)	10	5	0	5,693	0	0	5,698	570
CADMIUM COMPOUNDS	10	904	1,391	5	0	0	2,300	230
STYRENE	10	4,724	636	5	0	7	5,372	537
COBALT	9	419	684	3,708	0	760	5,572	619
GLYCOL ETHERS	8	76,065	268,798	0	0	0	344,863	43,108
DICHLOROMETHANE	7	133,725	264,215	0	0	0	397,940	56,849
COBALT COMPOUNDS	6	18	781	535	0	3,100	4,434	739
CRESOL (MIXED ISOMERS)	6	6,341	1,801	258	0	0	8,401	1,400

The Mahoning River was polluted during the nineteenth and twentieth centuries by two major sources: the steel industries and the human population. Those industries released over 32000 Kgs of oil and grease per day in the river. Raw sewage from over 300000 residents was also a major contribution to the pollution of the Mahoning River. Estimates provided by the US Army Corps of Engineers explain the scope of the cleanup/restoration project (www.yzu.edu/mahoning_river). There are approximately

462,000 cubic yards of contaminated riverbed sediments. Along the shore in the banks, there is an additional of 286,000 cubic yards of contaminated sediments. This gives a total of 750,000 cubic yards of contaminated material spread out over 30 miles to be cleaned up (www.yosu.edu/mahoning_river). Heavy metals contamination is one of the major types of pollution found in the river sediment of the Mahoning River. Appearance of mutagenic and carcinogenic effects on fish and other wild life have been reported in studies. These adverse effects are attributed to organics such as polyaromatic hydrocarbons (PAHs), (OEPA Report, 1996, 2003, 2006 and 2007). PAHs contain fused aromatic rings and are products of coal, oil, gas, tobacco, and other organic substances burning. However the contribution of metals such as arsenic and lead can't be overlooked for these negative effects on water life.

1.4 Heavy Metals in soil sediment and their sources

Metals are naturally present in soil sediments. Various metal elements can be found in very minute quantities, but the soil contaminated with a pollutant may contain very high levels that can be hazardous to many forms of life in earth. They have a very high tendency to get adsorbed on to the soil particles or the sediments and thus increasing the concentration (Singer, 1972). The association of these elements with particulate and colloidal species can be attributed to a variety of mechanisms. The larger sand and silt particles consist of a variety of minerals, within which are incorporated many of these elements. Clay minerals, also contribute large quantities of heavy metals. The mechanisms by which these particles carry and contribute heavy metal to a stream include sorption and ion exchange onto the mineral surface, as well as incorporation into its structure. The sorption of these elements onto such mineral surfaces can occur without

ion exchange as a result of coulombic interactions with the surface in its electric double-layer. Most colloidal or particulate matter in water has a negatively charged surface and can attract positively charged metal ions (Vanoni, 1975). The most abundant heavy metals in nature include iron (Fe), manganese (Mn), zinc (Zn), lead (Pb), copper (Cu), chromium (Cr), aluminum (Al), and cadmium (Cd).

There are basically two sources of pollution, point and non-point sources. Discharged from one particular location or point, such as an industrial plant, are referred to as point sources. Non-point sources are devoid of any particular or discrete location, such as runoff from agricultural lands, or atmospheric deposition (www.epa.gov). The main sources of heavy metals include (Singer, 1972),

- Coal burned for energies
- Emission from coke ovens in the manufacture of metallurgical coke
- Automotive fuel burning and emission
- Emission from iron and steel manufacturing plants
- Municipal wastes and leached agricultural chemicals such as fertilizers
- Metal corrosion products

1.5 Hazardous metals, their sources and health impact (www.lenntech.com)

Barium

Barium enters the environment through mining, production of barium compounds, coal and oil combustion. Respiratory problems, high blood pressures, cardiac arrhythmia, bowel irritation, muscular weakness, swelling of brains, liver, kidney and heart damage are the major health effect of barium exposure.

Selenium

Selenium is released in the environment through agricultural, industrial activities, coal and oil combustion. It may cause respiratory problems, fever, headache, bowel irritation, eye irritation, skin irritation, brittle hair, deformed nails, and discoloration of nails, teeth and hair.

Silver

Silver is released in the environment through human activities such as smelting, hazardous waste sites, and cloud seeding with silver iodide, metal mining, sewage outfalls, and the photo processing industry. It may cause eye, skin and respiratory irritation. Drowsiness, staggering, confusion, unconsciousness, nausea, bowel irritation and narcosis may results from acute exposure. Chronic overexposure may cause kidney, eye, lung, liver, anemia, cardiac abnormalities and brain damage.

Lead

Lead enters the environment from leaded gasoline, fuel combustion, industrial processes, solid waste combustion, leaded pipeline, and leaded paints. Lead is one out of four metals that have the most damaging effects on human health. The major health impacts of the lead include anemia, rise in blood pressure, brain damage, miscarriages, CNS, kidney and sperm damage.

Arsenic

Human activities such as fossil fuel burning, volcanoes, methyl arsines released by microorganism are major source of environmental arsenic. Exposure to inorganic arsenic may cause gastro-intestinal irritation, reduced blood cells production, skin pigmentation, and lung irritation. It may also intensify the chances of skin, lung, and liver

and lymphatic cancer and may cause brain damage, miscarriages, heart disruptions and infertility.

Cadmium

Cadmium is released in the environment through weathering of rocks, forest fires and volcanoes and human activities. Cadmium may cause kidney damage, diarrhea, bowel irritation, bone fracture, reproductive failure, infertility, CNS damage, DNA and immune system and psychological disorders.

Chromium

Chromium enters the environment through natural processes and human activities such as coal combustion, steel, leather and textile and electroplating industries. It can produce skin rash, nose irritation, stomach upsets, respiratory problems, weakened immune systems, kidney and liver damage, lung cancer and sometimes even death.

Mercury

Mercury enters the environment through natural process such as breakdown of minerals and rocks and through human activities such as fossil fuels combustion, mining, smelting, solid waste combustion and agricultural fertilizers. Microbial activities can convert mercury to methyl mercury which may quickly get absorbed in the organisms causing severe brain damage. They may also cause kidney damage, intestine damage, lung irritation, skin irritation, CNS damage, reproductive failure and DNA alteration in the animals.

1.6 Heavy metals bioremediation in soil sediment

Heavy metal pollution creates environmental problems due to their high toxicity. Soils contaminated from industrial effluents, and other sources contain large

concentrations of heavy metal, that present serious ecological and health problems due to their accumulation in the food chain. Metal removal from the soil sediment using common physico-chemical techniques such as oxidation and reduction, chemical precipitation, filtration, electrochemical treatment, evaporation, ion-exchange and reverse osmosis processes is limited because they are too expensive. The use of metal resistant microorganisms along with different carbon sources is an alternative for the removal of these toxic contaminants. Bioremediation is a technique that uses various microbes and plants for the detoxification of hazardous substances such as metals. It consists of a series of applications that detoxify sediment without transferring hazardous substances from one medium to another. This technique can also be utilized onsite and is less expensive than physico-chemical techniques. Bioremediation with microbes involves metal-microbial interactions to concentrate and remove. Microbial interactions include binding of metal to the cell or cell surface, translocation within the cells, biotransformation within the cell, and volatilization or precipitation in the form of salts by interactions with extracellular components and anions produced by microbes (Brierly, 1990). Bioremediation also greatly increases metal flux and mobilization from the solid phase facilitating removal processes (Amezcuca & et al., 2005). Various studies showed that fungi are useful in removing metals from soils and sediments. The cell wall of fungi is composed of polysaccharides, proteins and lipids with functional groups that are capable of chelating or binding metals. *Pleurotus ostreatus*, a white-rot fungi, can uptake metal and concentrate in their mycelia (Favero, 1991). *Pleurotus ostreatus* were able to concentrate up to 20 mg.g⁻¹ of cadmium from a liquid media containing 150 ppm of cadmium (20% deposited intracellularly) (Favero, 1991). *Phanerochaete chrysosporium*,

another species of white-rot-fungi was found to have sorption capacities for metals such as Cd, Cu, Hg, Ni and Pb. In one study, approximately, Ni, 56 mg.g⁻¹; Hg, 61 mg.g⁻¹; Cu, 60 mg.g⁻¹; Pb, 108 mg.g⁻¹ and Cd, 110 mg.g⁻¹ were found to be absorbed by fungi mycelia (Baldrian, 2003). Fungi are also capable of mobilizing metal from the sediment deposits. A study conducted with a fungal species *Penicillium frequentans*, showed that metal fluxes increased from <0.1 pgcm⁻² s⁻¹ before bioremediation to between 0.2 and 0.5 pgcm⁻² s⁻¹ after bioremediation. Metal concentrations in the soils solution increased from 2 - 10 µg l⁻¹ (Cu), 1 - 4 µg l⁻¹ (Pb) and from 40 - 140 µg l⁻¹ (Ni) with this fungus after bioremediation (Amezcuca & etal, 2005). It indicates that metals can become more bio-available after remediation due to increased mobility out of the sediment. Some other species such as *Pseudomonas spp.*, *Anthrobacter spp.*, and *Klebsiella spp.* can also be used for remediation purpose because of their ability to produce bio-surfactants. Bio-surfactants are biodegradable molecules that do not impose any harm to the environment (Miller, 1995). They facilitate mobilization of metals from sediment deposits due to their surfactant properties.

CHAPTER 2

INSTRUMENTATION, METHODS AND PROCEDURES IN BRIEF

2.1 Inductively Coupled Plasma- Atomic Emission Spectroscopy

Concentration of extracted metals was determined using an Inductively Coupled Plasma – Atomic Emission Spectrometer (ICP-AES), Thermofischer iCAP 6500 Duo. Cold vapor technique with Perkin Elmer FIMS AS90 mercury analyzer was used to analyze mercury.

Atomic emission spectrophotometry is a technique in which electrons in the atoms are excited at high temperature. The excited atom emits energy at a given wavelength as it returns to its ground state. The Intensity of emitted energy is proportional to the amount of the element ($I \propto C$). It involves three basic steps: atom formation, excitation and emission. ICP-AES utilizes plasma as the atomization and excitation source.

Plasma is an electrically neutral, highly ionized gas that consists of ions, electrons and atoms. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) determines trace elements, including metal, in solution. Detection limits, sensitivity, and optimum and linear concentration ranges of the elements can vary with the wavelength, spectrometer, matrix and operating conditions. Samples must be solubilized or digested in appropriate solvents before analysis.

ICP-AES measures characteristic emission spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element-specific emission spectra are produced by radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the emission lines are monitored by photosensitive devices. Background correction is required for

trace element determination. The background must be measured adjacent to analyte lines on samples during analysis.

Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. Table 2-1 shows the wavelengths for individual element with minimum multielement interferences, method detection limit (MDL) and reporting limit (RL) in this study. The MDL is a value generated statistically, which refers to the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The RL represents a practical and routinely achievable quantitation limit at > 99.9% confidence.

Table 2-1. Wavelengths (nm), method detection limit and reporting limit for ICP analysis of different elements.

Metal	Wavelength	MDL($\mu\text{g/L}$)	Reporting limit($\mu\text{g/L}$)
Ag	328	1.87	10
As	193.7	4.13	10
Ba	455.4	2.42	10
Cd	214.4	1.10	10
Cr	267.7	0.38	10
Hg	253.7	0.07	0.2
Pb	220.3	1.02	10
Se	196	4.08	10

2.2. X-Ray Fluorescence Spectroscopy

When a sample material is bombarded with X-Rays, it gets excited and releases X-Ray fluorescence. The material emits energy characteristic of the atoms involved. The absorption of higher energy results in the release of lower energy emissions; hence the term “fluorescence” is used. In most cases the innermost K and L shells are involved in the process of XRF detection hence X-Ray emission lines are commonly referred to KA, KB, LA or LB. In energy dispersive system, the spectrometer focuses all the emitted X-rays onto an energy analyzing detector.

In this experiment, due to the presence of carbon components in samples, liquid extracts were used for analysis to avoid possible interferences in instrumental analysis. A previously established ‘water’ method, was provided by technical support from Bruker Corporation was used. The liquid samples were placed into the closed sample cups and analyzed with 8 and 30 minute instrument run times.

2.3. Toxicity Characteristic Leaching Procedure

2.3.1. Overview

The TCLP was intended to be a laboratory test designed to simulate leaching in a municipal landfill. In 1982, as an employee of the USEPA, Todd Kimmell played a key role in the development of the TCLP. He described the TCLP as the second generation of leaching tests at USEPA, replacing the Extraction Procedure (EP) (www.epa.gov). The TCLP was developed in response to the 1984 Hazardous and Solid Waste Amendments (HSWA), which directed USEPA to examine the EP and make changes to ensure that it accurately predicts leaching potential of wastes. The Resource Conservation and Recovery Act (RCRA) directed USEPA to establish characteristics that identify wastes that pose a threat when improperly managed. One of the characteristics established was

the Toxicity Characteristic (TC), which was developed to identify those wastes, which when improperly managed might result in contamination of ground water. Scientists from USEPA and Oakridge National Laboratory did extensive research and developed the method known as TCLP that not only retained the EP's batch extraction format as the basic mode of the test, but also employed an acetate buffer leaching fluid to simulate the effect of decomposing municipal waste. The method was shown to be more accurate than the EP and other available laboratory leaching tests and was suitable for assessing the leachability of organic compounds that met the HSWA mandate (www.epa.gov). The TCLP is designed to determine the mobility of both organic and inorganic analytes present in liquid, solid, and multiphasic wastes. This is usually used to determine if a waste may meet the definition of EP Toxicity, that is, carrying a hazardous waste code under RCRA (40 CFR Part 261) of D004 through D052 (www.epa.gov).

In real landfill conditions, water and other liquids percolate over time which can often react with the solid waste and may possess environmental and health risks. Analyses of TCLP extracts determine which of the contaminants identified by the USEPA are present in the leachate and at what concentrations (www.epa.gov).

2.3.2. Scope and Application (Method 1311)

- a. The TCLP is designed to determine the mobility of both organic and inorganic analytes present in liquid, solid, and multiphasic wastes.
- b. If a total analysis of the waste demonstrates that individual analytes are not present in the waste, or they are present at low concentrations (< regulatory levels), the TCLP is not performed.

- c. If an analysis of any one of the liquid fractions of the TCLP extract indicates that a regulated compound is present at such high concentrations that, even after accounting for dilution from the other fractions of the extract, the concentration would be above the regulatory level (Table 2-1) for that compound, then the waste is hazardous and it is not necessary to analyze the remaining fractions of the extract.
- d. If an analysis of extract obtained using a bottle extractor shows that the concentration of any regulated volatile analyte exceeds the regulatory level for that compound, then the waste is hazardous and extraction using the zero head space extractor is not necessary. However, extract from a bottle extractor cannot be used to demonstrate that the concentration of volatile compounds is below the regulatory level.

Table 2-2. TCLP Regulatory limits for some hazardous materials (www.epa.gov)

EPA Hazardous Waste code	Contaminant	Regulated Level (mg/l) (or ppm)
D004	Arsenic (As)	5.0
D005	Barium (Ba)	100.0
D006	Cadmium (Cd)	1.0
D022	Chloroform	6.0
D007	Chromium (Cr)	5.0
D026	Cresol	200.0
D008	Lead (Pb)	5.0
D013	Lindane	0.4
D009	Mercury (Hg)	0.2
D036	Nitrobenzene	2.0
D038	Pyridine	5.0
D010	Selenium (Se)	1.0
D011	Silver (Ag)	5.0

2.4. Microwave Assisted Acid Digestion

2.4.1. Overview

To measure heavy metals in soil, sediment and waste, they need to be digested and dissolved before the measurement is carried out for their concentrations. Both ‘total’ and ‘partial’ digestion can be carried out for environmental monitoring. A suitable microwave assisted acid digestion method can provide analytical recovery of at least 90% of the soil bound elements. Nitric acid is capable of destroying organic matter and oxidizing sulphide materials. Therefore a complete decomposition of carbonates or carbon based samples is achieved.

2.4.2. Scope and Application

Microwave assisted acid digestion of sediments is applicable for the following elements:

Table 2-3. Elements applicable for microwave digestion (Method 3051)

Aluminum	Cadmium	Iron	Molybdenum	Sodium
Antimony	Calcium	Lead	Nickel	Strontium
Arsenic	Chromium	Magnesium	Potassium	Thallium
Boron	Cobalt	Manganese	Selenium	Vanadium
Barium	Copper	Mercury	Silver	Zinc

The method is intended to provide a rapid multi-element acid leach digestion.

2.5. Moisture and Dry Matter content

Moisture content is the total amount of water present in a unit weight of sediment sample. It is determined by drying the sediment in an oven. Hence it is also sometimes referred to as oven dry moisture content. Approximately 10 g of sediment was transferred

to a dish, weighed, and then dried in an oven at 105°C for 24 hours. The sample was placed in a desiccator for at least 3 hours to allow for temperature equilibration. The sample was then weighed for dry weight. Percent moisture content was then calculated.

2.6. Ash Value

Ash value expressed as the percentage of residue remaining after dry oxidation of the sediment (oxidation at 550 to 600°C). Ash value is an estimate or measure of the mineral content and other inorganic matter in the sediment. It may also contain various other materials such as hard and soft woods, herbaceous materials, agricultural residues, wastepaper, biomass and other residues that account for its weight. All results are reported relative to the 105°C oven-dried weight of the sample.

The dried samples contained large particles or chunks; each was ground to reduce the particle size to less than 1 mm in diameter. The sample was then re-dried at 105°C. The sample pan was dried initially to a constant weight by igniting at $575 \pm 25^\circ\text{C}$.

Approximately 5 g, to the nearest 0.1 mg, were weighed into crucible, placed into the muffle furnace and then ignited at $575 \pm 25^\circ\text{C}$ for a minimum of 24 hours. The crucible was then cooled in a desiccator and weighed to the nearest 0.1 mg. The crucible was heated repeatedly for one hour interval until the final weight was constant to within 0.3 mg. The final weight of the ash was recorded and reported as percentage.

2.7. Particle Size Analysis

Soil particle size analysis determines the amount of each particle size group (sand, silt or clay) present in the sediment. Sand is the largest soil particle size [2.0 mm-0.05 mm], silt is the intermediate [0.05 mm- 0.002 mm], and clay is the smallest [< 0.002 mm] (US Department of Agriculture). Particles of size greater than 2 mm are called stones, rocks or

gravels and are not considered to be soil material. When a mixture of soil is suspended in a water column, the heaviest particles settle immediately. Sand settles after 2 minutes, silt after 24 hours and clay remains in suspension.

In this procedure, the oven dried sediment was ground to fine particles and sieved [No. 200 (0.074mm)]. Approximately 50 g of the sediment was soaked in 125 ml of solution containing 5% by weight of calgon (Sodium hexa meta phosphate) overnight. The soaked sediment was then transferred to a 1 L cylinder and the volume was made up to 1L by adding deionized water. The content was shaken and a wine/alcohol hydrometer was dipped into it. The hydrometer reading was taken at specified interval. The final reading was taken at 24 hour.

Table 2-4: Hydrometer and Temperature readings for Particle size analysis

Incubation	Time	Temperature ⁰ C	Hydrometer calibration	Hydrometer readings
Incubation 1	1 min	22	15.66 ⁰ C	1.02
	2 min	22		1.02
	24 hr	22		1.002
Incubation 2	1 min	21		1.02
	2 min	21		1.02
	24 h	21		1.002
Incubation 3	1 min	22		1.022
	2 min	22		1.02
	24 h	21		1.003

2.8. Bio-treatment and Incubation

The samples were treated with *P.ostreatus*, sawdust, paper and cyclodextrin as shown in table 2-5.

Table 2-5. Sample Bio-treatment protocol

Sample No.	Treatments
1	Control (sediment only with no treatment)
2	Sediment + Sawdust
3	Sediment + Paper
4	Sediment + Fungi +Sawdust
6	Sediment + Paper + Fungi
7	Sediment + Sawdust + Fungi + Cyclodextrin

On day 0, 1L of sediment was hand mixed with the treatments in separate fish bowls and was incubated at 25°C. Approximately 75 g of sediment from each bowl were taken for physical and chemical analysis. Further sampling was done on day 21 and day 42. Detailed protocols and procedures for each treatment are given in Appendix 3.1.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Metal content in TCLP extract using ICP-AES analysis.

In TCLP extracts, only barium, chromium and lead were detected in ICP-AES analyses. Other metals such as Ag, As, Cd, Hg and Se were not detected.

Barium

The concentrations of Ba in all samples were below the regulatory limit of TCLP. On Day 0, the leaching characteristics of Ba was reduced slightly in both samples, treated with sawdust and sawdust plus fungi (Figure 3-1). This may be because of the dilution of analytes by the addition of sawdust and fungi. On day 42, in a sample treated with sawdust and fungi, showed increased concentrations of Ba.

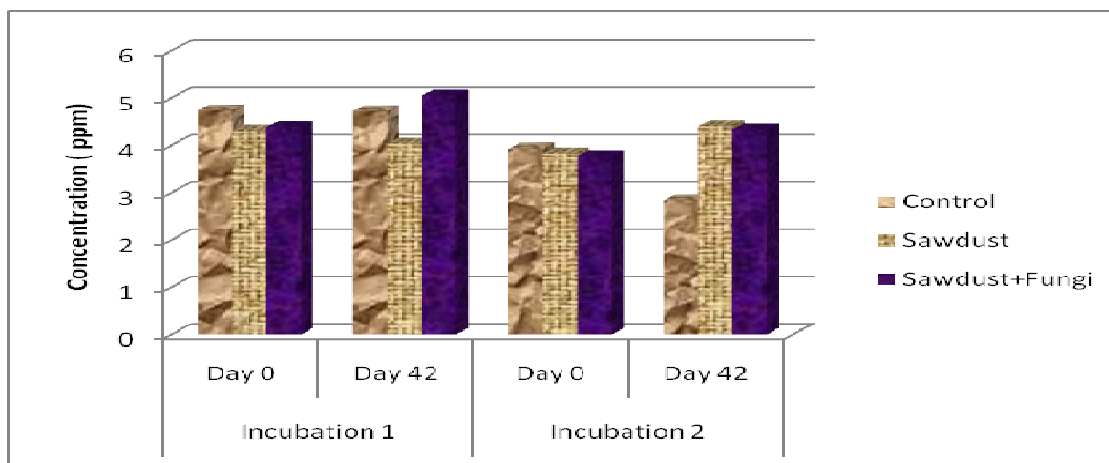


Figure 3-1: Toxicity Characteristics Leaching Procedures (TCLP) for Barium in Mahoning River sediment. Concentrations are shown for barium in TCLP extracts from control (sediment only), sawdust (sediment treated with sawdust only) and sawdust+fungi (sediment treated both with sawdust and fungi) from two separate incubations (1&2) initially and after 42 days.

Chromium

Overall, Cr concentrations in all samples were found to be below regulatory limit. On Day 0, the leaching characteristic of Cr followed similar trends as shown for Ba, i.e. slight decreases in values for samples, treated with sawdust and sawdust plus fungi (Figure 3-2). On day 42, in a sample treated with saw dust and fungi the leaching characteristics was found to be increased in both the incubations.

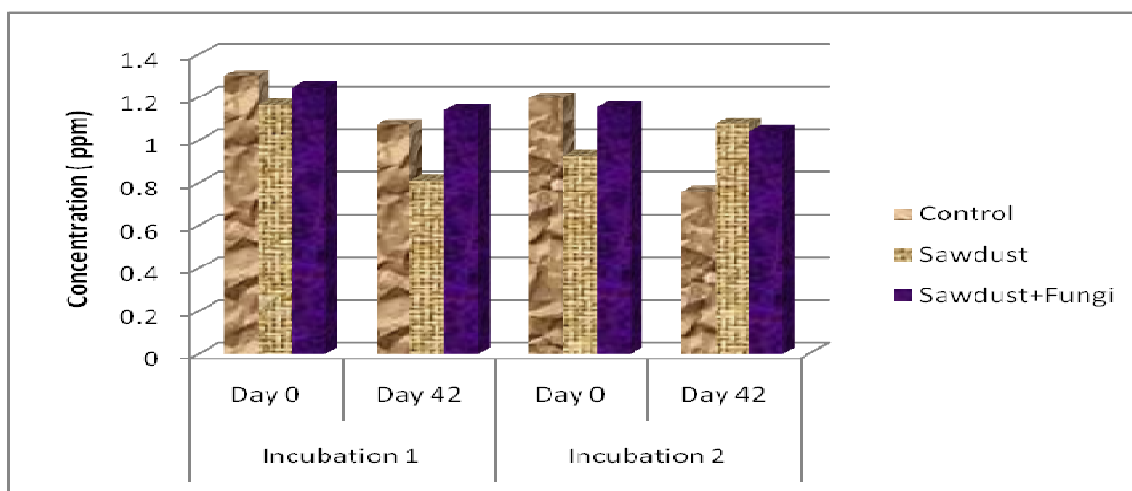


Figure 3-2: TCLP for Chromium. Treatments were as described for Figure 3-1.

Lead

The levels of lead in most samples were found to be closer to the regulatory limit. Mixed trends were observed for Pb in incubation 1 on Day 0. Pb concentration increased in some treatments and decreased in others (Figure 3-3). This may be due to sample heterogeneity. On day 42 / incubation 2 there was a significant reduction (30 – 50 %) in the leaching characteristics for samples treated with sawdust and sawdust plus fungi.

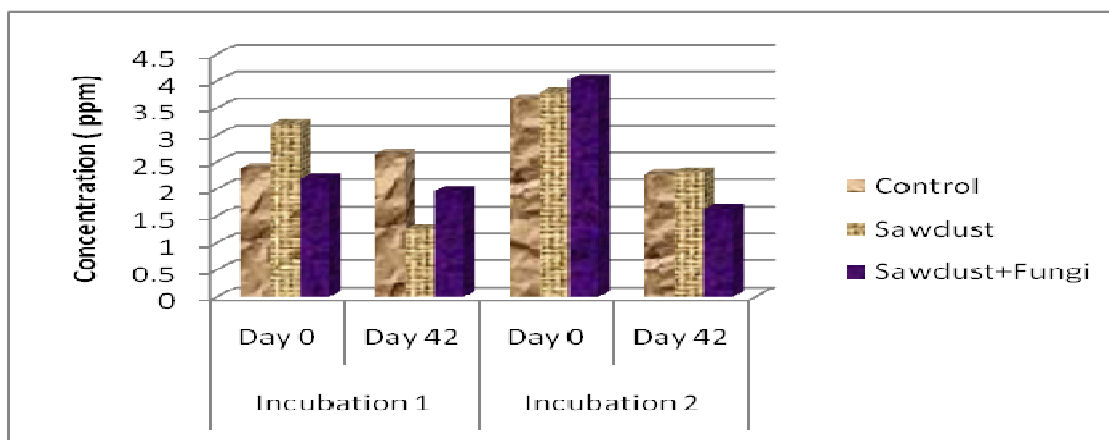


Figure 3-3: TCLP for Lead. Treatments were as described for Figure 3-1.

3.2 Overall TCLP Discussion

The fungal remediation in Mahoning River sediment showed some impact on leaching characteristics of few analytes. Metals such as As, Cd, Hg and Se were not detected but there were some changes in leaching observed with Ba, Cr and Pb. The changes were more pronounced on Day 42 of the treatment with fungi plus sawdust. The leaching of Barium increased by 50% in incubation 2. The leaching of Chromium increased by 30% in one of the incubations. The leaching of lead showed a 35% reduction. *Pleurotus ostreatus* was found to have some role in metal mobilization in the sediment as indicated by Barium and Chromium result. *Pleurotus ostreatus* might have also absorbed some metal from the sediment making it less available for leaching as seen with Pb. However, to confirm the trends observed, more samples and metal analysis in fungal biomass is required.

ICP-AES analysis was used for most RCRA metals: Ag, As, Ba, Cd, Cr, Pb and Se. The TCLP extracts were digested with HNO₃/HCl following method 3010 A, prior to analyses. The multielement interference were reduced by selecting appropriate wavelength with reduced elemental interference. For Hg analysis, the USEPA method

245.1 was used. Since organic Hg might exist in the sediment, digestion of the sample with potassium permanganate and potassium persulfate prior to analysis was necessary to convert organic Hg to elemental Hg. Hg was analyzed with a Perkin Elmer Mercury analyzer which make uses of cold vapor atomic absorption techniques.

3.3 Metals content in Microwave Assisted Total Acid Digestion sediments (TAD) using ICP-AES.

Silver

In incubation 1 and 2 at day 0, the concentration of silver was reduced slightly with both treatments (Figure 3-4).The dilution of the samples with sawdust and fungi accounts for the reduction in concentrations. In both incubations on day 42, there was a slight decrease in the concentrations of silver for both treatments.

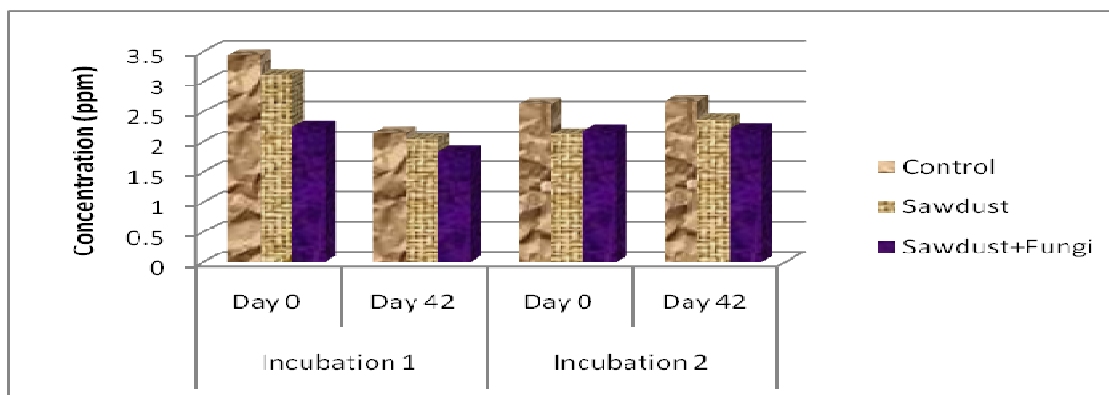


Figure 3-4: Total Acid Digestion (TAD) of Silver.

Concentrations are shown for Silver in Total acid digests from control (sediment only), sawdust (sediment treated with sawdust only) and sawdust+fungi (sediment treated both with sawdust and fungi) from two separate incubations (1&2) initially and after 42 days.

Arsenic

For arsenic, there was not a clear trend on day 0 with increase and decrease in concentrations. However on day 42, a significant reduction in the concentration was observed for both incubations (Figure 3-5) and for both treatments.

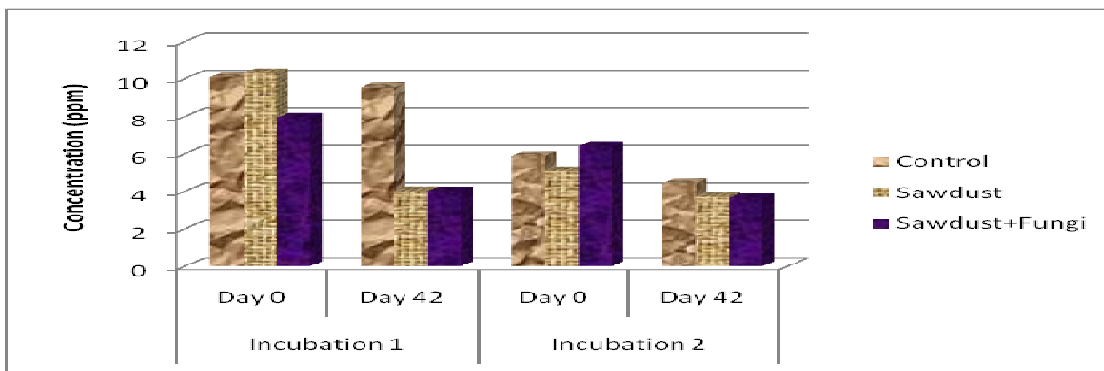


Figure 3-5: TAD of Arsenic. Treatments were as described for Figure 3-4.

Barium

For Barium, there was decrease in concentrations on day 0. On day 42, a more significant reduction in the concentration was observed in incubation 1 and slight reduction in incubation 2 (Figure 3-6). This trend was observed for both treatments.

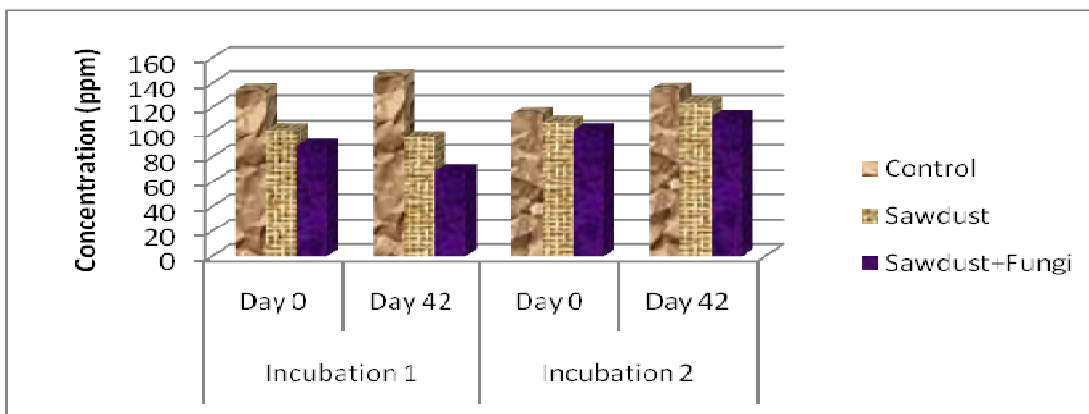


Figure 3-6: TAD of Barium. Treatments were as described for Figure 3-4.

Cadmium

The trend for cadmium was similar to that for Barium. There was a significant reduction in the metal concentration on day 42 for incubation 1 and only a slight decrease was seen with incubation 2 (Figure 3-7). A reasonable decrease in concentration was seen on day 0. It is possible that fungi might have started absorbing as soon as it comes in contact with the metal.

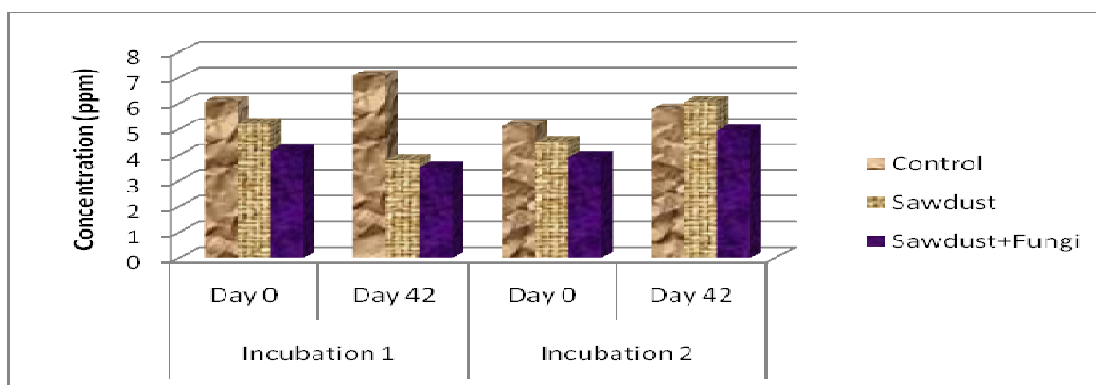


Figure 3-7: TAD of Cadmium. Treatments were as described for Figure 3-4.

Chromium

Chromium followed similar trend as that of Cadmium. Reasonable reduction in concentration was observed even at day 0 and large reduction were observed at day 42 (Figure 3-8).

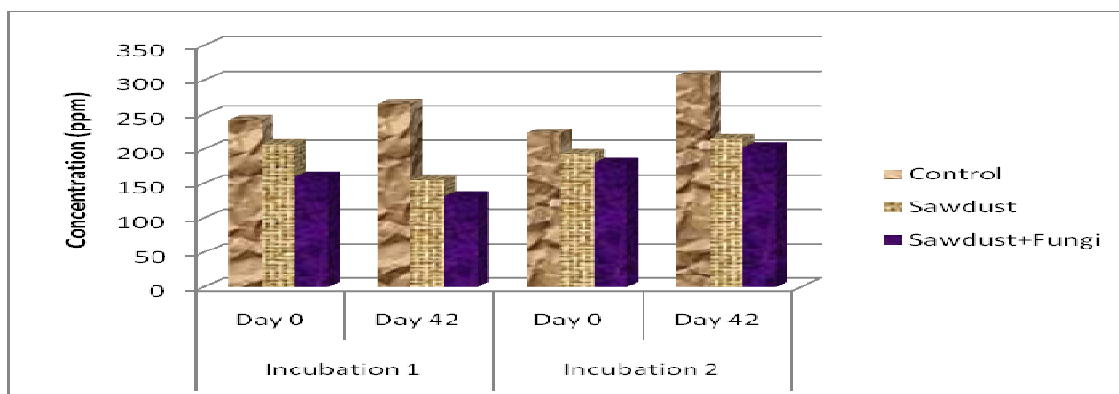


Figure 3-8: TAD of Chromium. Treatments were as described for Figure 3-4

Lead

A large reduction in the concentration was observed in day 42 with Incubation 1 (Figure 3-9). In incubation 2 the concentration almost remained same in day 0 and day 42 samples.

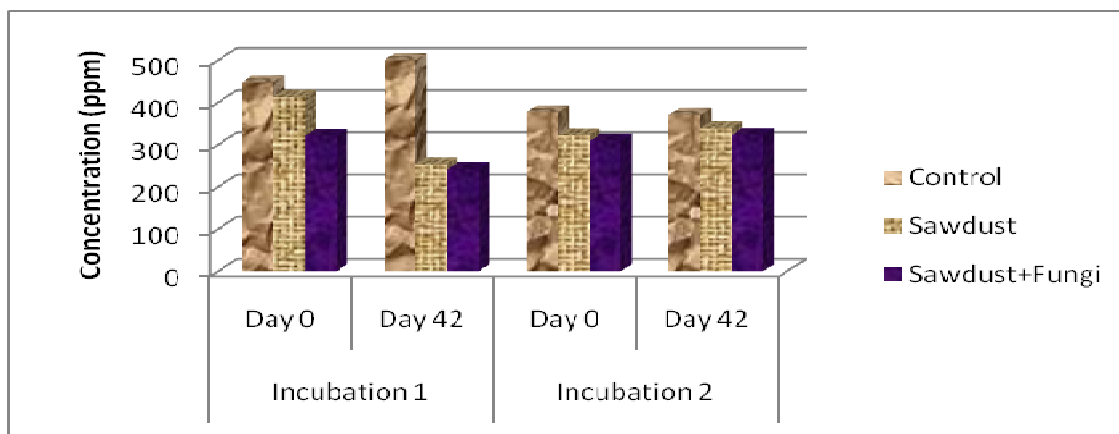


Figure 3-9: TAD of Lead. Treatments were as described for Figure 3-4.

3.4 Overall TAD discussion

Unlike TCLP, in total acid digestion, total metal available for leaching is determined. The concentrations of all elements detected, on untreated samples were above the Region 5 RCRA ecological screening levels (ESLs). The ESLs is a risk

assessment tool that determines adverse risk to the environment. It represents a protective quality benchmark for 223 contaminants and 4 environmental media i.e. air, water, sediment and soil (www.epa.gov). ICP-AES technique was utilized for the analysis. The multielement interference were reduced by selecting appropriate wavelength with reduced elemental interference.

In both incubations on Day 0, the concentrations of most of the elements were reduced slightly except for Cd and Cr where the reduction was more pronounced. Dilution of the samples with sawdust and fungi accounts for the reduction in concentrations of all elements. However, the lag period between the sampling and extraction might have also contributed to the reduced concentrations at Day 0. It is difficult to rule out the possibility of fungi acting on the substrate even on day 0. Since all results are based on dry weight, further data interpretation may be required using ash weight. There was a remarkable decrease in the concentrations with treatments for most of the elements on day 42, especially with the incubation 1. Typically there was approximately 40-50% reduction in the content of metals such as As, Ba, Cd, Cr and Pb observed at day 42 with incubation 1 in samples treated with sawdust and fungi. The Ag content was reduced by ~ 15%. There was also reduction in the element's concentration in a sample treated with sawdust only. Although no fungus was added to it, some growth was observed to certain level.

3.5 Metal contents using XRF

Samples were analyzed by a 'water' method set for liquid samples as recommended by Bruker Corporation for S2 ranger XRF machine, with a scan time of 8 and 30 minutes. Due to the corrosive nature of samples, they were placed in sample cups

closed at both ends with Mylar plastic film. To find out the sensitivity of the method and instrument, original standards with a concentration of 20 ppm for most elements were analyzed first. The best results were obtained for Zn with a variation of about $\pm 5\%$. For the elements, As, Ba, Cu, Se, Mo, Cd, Hg, and Pb variations of about $\pm 35-50\%$ were observed and for the elements such as Sn, Sb, and Ag variations up to $\pm 1000\%$ were observed. For lower concentrations (0.5 – 5 ppm), many elements that showed up in the previous analyses did not appear at all and those that did appear showed variations up to 1000%. With the actual samples, many elements were not detected. The reproducibility was very poor, hence further analyses using XRF was discontinued.

3.6 Moisture Content

Moisture contents were determined for all samples treated with sawdust, fungi, paper, surfactant, nitrogen and some combinations of these treatments. Moisture content remained almost same throughout the incubation over all treatments. Moisture content was reduced over time affecting microbial activity. To overcome this issue, a small quantity of water was placed in a petri dish, inside the glass bowl. The glass bowls were sealed with a plastic wrap before placing them in the incubator. Since the moisture content were found to be uniform throughout the incubations, any change in microbial activity due to change in moisture environment can be assumed as negligible. The moisture contents ranged from 40-45% for all treatments [$\pm 0.5 - 2\%$ standard deviation] (Figure 3-10). Relatively high moisture content was present in control and in a sample treated with cyclodextrin. This might have accounted for the increase in total weight to moisture ratio. Low moisture content observed on sample treated with sawdust and fungi and sawdust only.

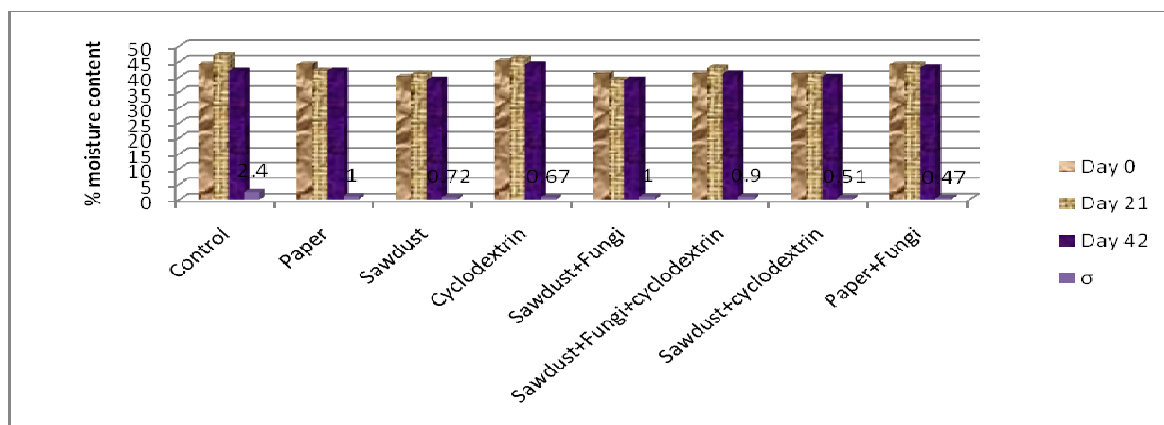


Figure 3-10. % Moisture content.

Moisture contents in sediments on day 0, day 21 and day 42 in three incubations in control and sample treated with paper, sawdust, surfactant and fungi.

3.7 Ash content

Ash contents were determined for samples treated with sawdust, fungi, paper, surfactant, nitrogen and some combinations of these treatments. Similar trends as for moisture contents were observed for ash weight. Ash content remained almost same throughout the incubation for all treatments. The ash contents ranged from 68-84% for all treatments with a standard deviation of $\pm 0.5-3.5\%$ (Figure 3-11). Relatively high ash content was found in a control and in a sample treated with surfactant. Low ash content was observed in samples treated with sawdust and fungi and sawdust only.

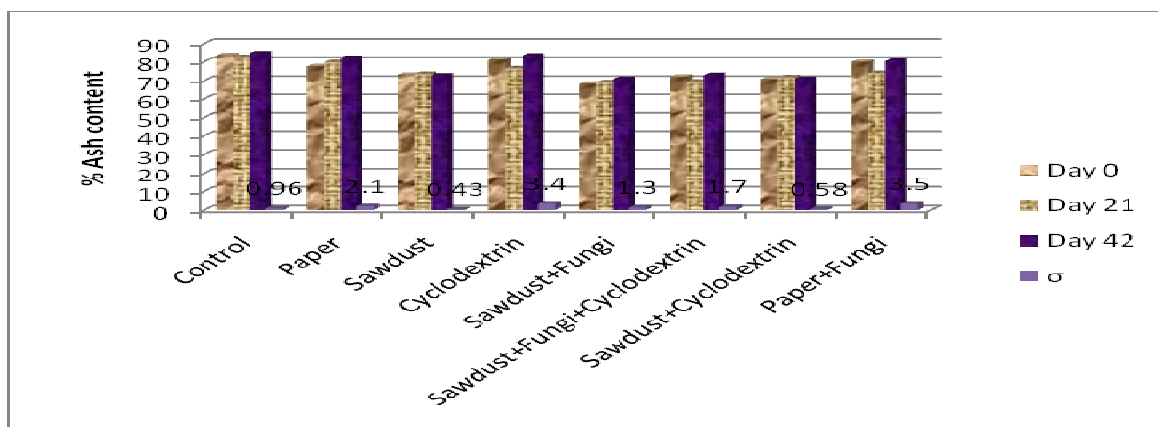


Figure 3-11: % Ash weight.

Average ash contents of a sediments on day 0, day 21 and day 42 (with standard deviation) in three incubations that were untreated (control) and treated with paper, sawdust, surfactant, fungi and some combination of these.

3.8 Particle size Analysis

Particle size is the most significant property of sediments. The particle size analysis can be used to characterize sediment properties. Both physical and chemical compositions are dependent on particle size. The particle size distribution of Mahoning River sediment from the Lowellville site is as follows: 56.3% (particles from 0.05-0.002 mm), 38.7% sand (particle size from 2.00-0.05 mm), and 4.99% clay (particle size smaller than 0.002 mm), (Figure 3-12). The sediment particles were ground to fine particles that can pass through a 0.074 mm sieve before analysis. The combined Silt and sand (~ 95%) portion indicates that, the soil mostly contains inorganic components

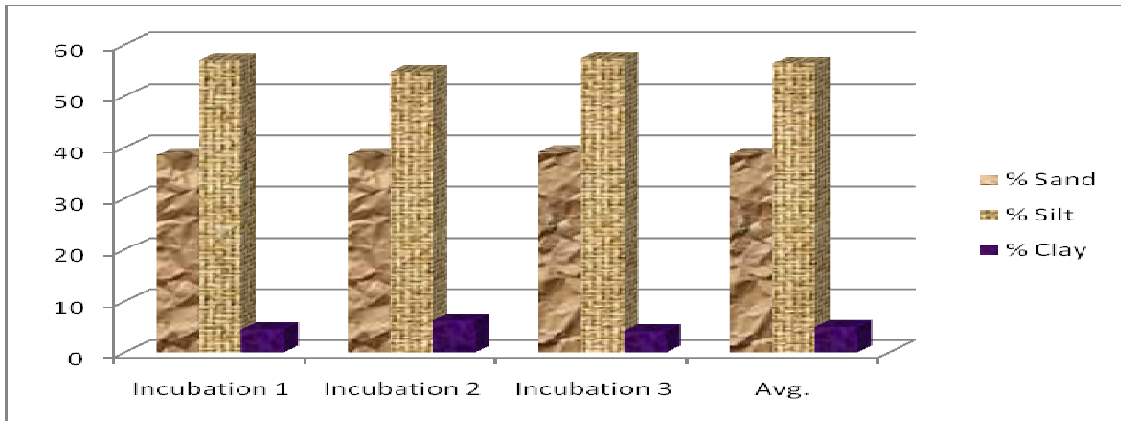


Figure 3-12: Sediment's composition in triplicate run.

Percentage of different particle sizes (sand, silt and clay) in sediment on day 0 in three incubations without treatment (control).

CHAPTER 4

SUMMARY, CONCLUSION AND RECOMMENDATION

4.1 SUMMARY AND CONCLUSION

Mahoning River sediment in Lowellville, OH is highly toxic from metal contaminants. The concentrations of most of the metals were above ecological screening levels as confirmed with the total metals study. Most of the TCLP metals detected were below the regulatory limit. Due to the aging and compaction of sediments the metals might have entrapped in organic and inorganic components within the sediment deposits inhibiting their leaching. The leaching of barium and chromium were increased with fungal treatment whereas for lead it was decreased. Fungal remediation may increase metal availability in the short term. Increased availability may allow more effective removal by plants. By changing the anaerobic sediment to an aerated soil, metal availability might decrease due to oxidation process. The concentrations of almost all metals were found to be reduced with sawdust and fungal treatments. *Pleurotus ostreatus* is shown to have effect on removing metals from the contaminated sediments. The most possible mechanism must be sorption. During this process, there is also a possibility of some metal's mobilization facilitating their leaching.

The findings of this study are expected to be helpful in future studies. Some other areas in which this study might be useful includes:

- It can serve as a baseline data for phytoremediation of metals from river sediment.
- Estimation of life in river.
- Role of microbial community in clean up process.
- Impact of industrial emission at other locations.

4.2 RECOMMENDATION

- Complete analyses and statistics with triplicate incubations data.
- Homogenous mixing of treatment with mechanical device and analysis from three different spots is recommended.
- *Pleurotus ostreatus* was found to be effective in removing metals from the contaminated sediment. However more controlled study with the fungi is recommended before making any solid conclusion.
- A controlled study with *Pleurotus ostreatus* is strongly recommended that can be done by incubating the fungi in a solution containing known amount of different metals.
- For triplicate incubations, sediments from the same site and spot are recommended and need to be sampled on same time.
- Nitrogen, cyclodextrin or other surfactant's treatments should be included for further studies on metal mobilization and availability.
- Bio-surfactant producing bacteria (e.g. *Pseudomonas* & *Klebsilla*) should be tested for metal mobilization.
- Determine transformation mechanisms for change in metal concentrations with treatments.
- Conduct study on the effects of pH on metal uptake by the microbial community.
- Scale up the technology.

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APPENDIX 1

1.1 Procedures for Chemical Properties

1.1.1 Toxicity Characteristic Leaching Procedure (Method 1311)

Two different buffered acidic leaching extraction fluids are used for TCLP depending on the alkalinity and the buffering capacity of the wastes. As described in TCLP, if the pH of the soils is less than 5, extraction fluid 1 with a pH of about 4.93 ± 0.05 (Add 5.7 mL glacial CH_3COOH to 500 ml of reagent water, add 64.3 mL 1 N NaOH, and dilute to a volume of 1 liter.) is used; otherwise extraction fluid 2 with a pH of about 2.88 ± 0.05 (Dilute 5.7 mL glacial CH_3COOH with reagent water to a volume of 1 liter.) is used. The pH values of the two solutions are adjusted with 1 mol/L HNO_3 and 1 mol/L NaOH. An aliquot of 50.00 g of each sample and 1000 mL extraction reagent were transferred into an extractor bottle which was then closed tightly by sealing with Teflon tape. It was then secured in a rotating agitation device, and rotated at 28 to 32 rpm for 18 ± 2 h with a speed of 30 ± 2 rpm. At the end of the 18-h extraction period, fluid in each vessel were separated from the solid phase by vacuum-filtration through micro glass fiber filter paper. The pH of the TCLP extracts was then measured and all extracts were acidified with 1 N HNO_3 to a pH of less than 2 for long-term preservation. The extracts were digested with HNO_3/HCl following Method 3010A prior to the ICP-AES analysis. The concentrations of heavy metals in extracts produced by TCLP were determined using ICP-AES. For mercury analysis cold vapor technique was used after digesting with potassium permanganate and potassium persulfate following Method 245.1.

1.1.2 Microwave Assisted Acid Digestion

Since ICP is capable of analyzing the trace metals in liquid only, the sediment sample could not be used directly. The term “digestion” refers to extraction of trace metal from sediment into aqueous form. There are different combinations of acids that can be used for digestion. For our samples, concentrated nitric acid is chosen.

The following steps are used for digestion:

1. The dry sample was ground with mortar and pestle and about 1 g of it was weighed and placed in a fluorocarbon (TFM) microwave vessel.
2. 20 ml of 1:1 (v/v) conc. HNO₃: H₂O was then added to the vessel and capped by placing safety valve.
3. The vessel with sample was placed in the turntable and was attached with venting tube.
4. Steps 1 to 3 were repeated until the turntable contains 3 vessels.
5. The MDS-81D exhaust was turned on to the maximum fan speed and the turn table was activated so that it was rotating.
6. The instrument was then programmed for 10 and 50 minutes in program 1 and 2 respectively with corresponding 75% and 55% power scheme. The START key was then depressed, allowing the sample mixtures to heat.
7. After the elapsed time, vessels were allowed to cool to a room temperature and vented manually.
8. The samples were completely transferred to a Fischer® conical centrifuge tube (de-ionized water is used to ensure complete transfer) and covered with Parafilm®.

9. The tubes were centrifuged at 5000 rpm for 10 minutes and the supernatant liquid was transferred to a 100 mL volumetric flask after filtration through Whatman glass micro fiber filter.
10. The volume was made up to the 100 mL mark with de-ionized water and the solution was transferred to a labeled Fisherbrand® polypropylene specimen container (with screw cap) for storage.

APPENDIX 2

2.1 Procedures for Physical Properties

2.1.1 Moisture Content/Dry Matter

Moisture content is the total amount of water present in a unit weight of sediment sample. Total moisture content is sometimes referred to as oven dry moisture content. The term “oven dry” refers to the laboratory method of obtaining the moisture content. Clean aluminum dishes were numbered and weighed (M_a). Approximately 10-15 g of sediment was scooped out, transferred to a dish, weighed (M_{a+s}) and then dried in an oven at 105 °C for 24 hours. The sample was placed in a desiccator for at least 3 hours to allow for temperature equilibration. The sample was then weighed to get the dried weight ($M_{a+dry\ Sed.}$).

Percent moisture content (MC) was calculated as:

$$MC = (M_{liq} / M_{Sed.}) \cdot 100$$

Where, M_{liq} = Mass of moisture = $M_{a+s} - M_{a+Dry\ Sed.}$

M_s = Mass of sediment = $M_{a+Sed} - M_a$

The term “dry matter content” in the sediment is given by

$$DM = 100 - MC$$

2.1.2 Ash Value (www.cobweb.ecn.purdue.edu)

Ash value expressed as the percentage of residue remaining after dry oxidation of the sediment (oxidation at 550 to 600°C), that may also contain various other materials such as hard and soft woods, herbaceous materials, agricultural residues, wastepaper, biomass and so many other residues that accounts for its weight. All results are reported relative to the 105°C oven-dried weight of the sample. It is an approximate estimate or measure of the mineral content and other inorganic matter in the sediment.

The dried samples contained a large particles or chunks; it was ground or milled to reduce the size of the large pieces to less than 1 mm in diameter. The sample is then re-dried at 105°C prior to testing. The sample pan was dried initially to the constant weight by igniting at $575 \pm 25^\circ\text{C}$.

A porcelain crucible was marked using a porcelain marker, placed into the muffle furnace, and heated to constant weight by igniting at $575 \pm 25^\circ\text{C}$ for three hour followed by cooling and re-heating for one more hour at the same temperature so that the final weight of the crucible varied by 0.3 mg from the previous weighing. This is recorded as the container's tarred weight. Approximately 5-6 g of oven dried sediments was weighed W_2 , to the nearest 0.1 mg, into the crucible and placed into the furnace and ignited at $575 \pm 25^\circ\text{C}$ for 24 hours.

The crucible is then removed from the furnace with its contents to a desiccator, cool to room temperature, weigh to the nearest 0.1 mg, and record this weight. Heating is repeated for one hour periods until the weight after cooling is constant to within 0.3 mg. The final weight of the ash was recorded as, W_1 , as the container plus ash weight minus container tare weight.

$$\text{Ash, \%} = (W_1/W_2) \times 100$$

Where, W_1 = weight of ash, and

W_2 = initial weight of 105°C dried sample.

2.1.3 Particle Size Analysis

The use of hydrometer in particle size analysis involved settling of particles, the velocity of which is measured by using Stoke's law:

$$V = ((\gamma_s - \gamma_w)D^2)/18\eta$$

Where, v = settling velocity (cm/s)

γ_s = Specific weight of soil solids (g/ cm³)

γ_w = Unit weight of soil (g/cm³)

η = Viscosity of water (g·s/cm²)

D = Diameter of the soil particle, cm

When a hydrometer is suspended in water where the soil is dispersed, it will measure the specific gravity of the soil-water suspension at a depth, L, called the effective depth. At time, t, from the beginning of the test, the soil particles that settle beyond the zone of measurement will have a diameter given by

$$D = A \cdot (L / t)^{1/2}$$

Where

D = particle diameter, mm

A = constant given by

$$A = ((30\eta) / (G_s - 1)\gamma_w)$$

L = Calibrated values depending on hydrometer reading, cm

t = time interval of hydrometer reading, min

G_s = Specific gravity of soil solids

The procedure used for particle size analysis (www.soil.gsfc.nasa.gov) was as follows:

- 1) A dry sediment sample was ground to fine particles with mortar and pestle.
- 2) The ground sample was sieved through a 0.074 mm (# 200) sieve. About 50 g of sediment passing through the sieve was stored in a suitable container.
- 3) A deflocculating agent (5% solution of sodium hexametaphosphate) was prepared by adding 50 g of calgon to 1 L of distilled water and mixing it thoroughly.
- 4) The samples were soaked in 125 mL of deflocculating agent overnight (12 hours).
- 5) A solution with 125 mL of deflocculating agent diluted to 1L by adding deionized water was prepared in a graduated cylinder. The wine/ alcohol hydrometer was placed in the solution to get the initial reading.
- 6) The soaked sample was stirred thoroughly and then transferred completely to a 1 L graduated cylinder, rinsing with a wash bottle.
- 7) Deionized water was added up to the 1 L mark and the suspension was shaken for the uniformity.
- 8) The cylinder was then set on table and the hydrometer was inserted slowly.
- 9) The reading on the hydrometer was recorded at intervals of 0.5, 1, 2, 4, 8, 12, minutes and 24 hour. The hydrometer was inserted 30 sec before the reading time.

APPENDIX 3

3.1 Bio-treatment and Incubation

On day 0, approximately 1L of sediment were added in each fish bowl from the bucket containing stock sediment collected from Mahoning river at Lowellville site. The sediment was premixed in a bucket before adding to the fish bowls. Sediment was added as per the protocol in table 2-5. Approximately 600 ml of sawdust were placed in bowl 2, 4 and 7 and approximately same volume (i.e. 600 ml) of paper was added in bowl 3 and 6. In addition, a culture of *Pleurotus ostreatus* was added in bowl No. 4, 6 and 7 and approximately 4.128 g of cyclodextrin (as a surfactant) in water solution was added in bowl No. 7. Samples were properly hand mixed with the treatments and approximately 75 g from each bowl were taken out for further physical and chemical analysis. The fish bowls were placed in the incubator (Percival, Iowa 50036) at 25°C.

On day 21, fish bowls were taken out of the incubator and half portion of the sample in each bowls were mixed. A clear boundary was marked to separate each zone. To the half portion of mixed half, that were treated with sawdust only, Fungi + sawdust and Fungi + sawdust + surfactant, a nitrogen source (25 ml) was added and mixed to facilitate fungal growth. Approximately 75 g of sample from each bowl and each zone (mixed or unmixed with or without nitrogen source) were taken out for physical and chemical analyses. While taking the samples, as much care was taken to get rid of white mycelia from the surface. The bowls were then placed in the incubator at 25°C. On day 42, final sampling was done from each bowl and each zone (Figure A3-1).

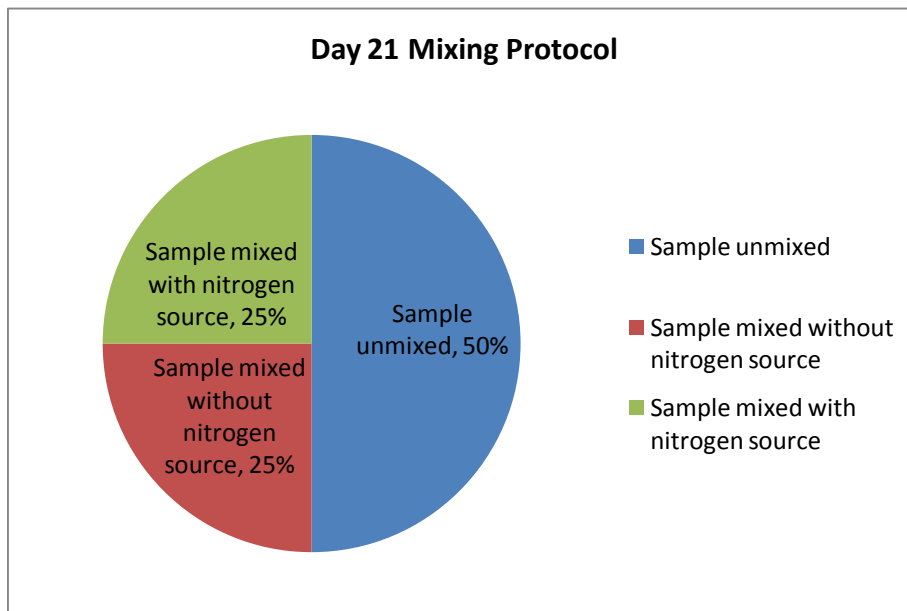


Figure A3-1. Mixing protocol for samples on day 21 with and without nitrogen source.

The circle represents fish bowl with three different zones in it.

APPENDIX 4

3.1 Inductively Coupled Plasma- Atomic Emission Spectroscopy

ICP-AES is an atomic emission spectrophotometric technique, based on the fact that excited electrons emit energy at a given wavelength as they return to ground state. The fundamental characteristic of this process is that each element emits energy at specific wavelengths characteristic to its chemical properties. Although each element emits energy at multiple wavelengths, the ICP-AES technique makes use of a single wavelength for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the analyzed sample. Thus, by determining which wavelengths and intensities are emitted by a sample, we can quantify the elemental composition of the given sample relative to a reference standard. ICP-AES utilizes plasma as the atomization and excitation source. Plasma is an electrically neutral, highly ionized gas that consists of ions, electrons, and atoms. The energy that maintains analytical plasma is derived from an electric or magnetic field. There is no combustion. Since the analytical plasmas operate with pure argon or helium, combustion is almost impossible. Plasmas are characterized by their temperature, as well as their electron and ion densities. Analytical plasmas typically range in temperature from 600 to 8000 K. As a comparison, the temperature of the sun's interior is millions of degrees, while its surface temperature is approximately 10000 K.

The ICP Torch

The ICP is radiofrequency-(RF, 27.12 MHz, and 40 MHz) induced plasma that uses an induction coil to produce a magnetic field (H). The ICP operates between 1 and 5 kilowatts. The induction coil is wrapped two or three times around the ICP torch and has

water flowing through it for cooling purposes. All ICPs have a capacitor bank that is continuously tuned to match the plasma's inductance. In order for the RF to travel along the surface of the hollow coil with minimum resistance, the coil is either gold or silver plated. Neither gold nor silver forms metal oxides upon contact with air. Although the RF power supply maintains the plasma, a tesla coil is used to ignite the plasma through the generation electrons and ions that couple with the magnetic field. The most common ICP torch in use today has evolved over decades of development (Figure A4-1). The circular quartz tube (12–30 mm OD) has three separate gas inlets. The only gas routinely used is argon. The gas enters the plasma through the outer channel with a tangential flow pattern at a rate of 8–20 Lmin⁻¹. The auxiliary gas, which travels up the center channel, also has a tangential flow (0.5–3 L min⁻¹) pattern. The nebulizer gas has a laminar flow pattern (0.1 to 1.0 Lmin⁻¹) and injects the sample into the plasma. The analytical zone is approximately 1 cm above the coils and offers the best optical viewing area for maximum sensitivity.

The plasma temperature in the analytical zone ranges from 5000–8000 K (the temperature varies with power, flow rate, etc.). The high temperature assures that most samples are completely atomized, although some molecular species (e.g., N₂, N₂⁺, OH, C₂, etc.) do exist and can be readily measured in the plasma. The plasma emits a continuum of background radiation that extends from the visible into the ultraviolet region (Figure A4-2). The radiation originates from electrons, Ar and Ar⁺, as well as various atomic and molecular species in the matrix. Although sample blanks can help offset the unwanted flux of background photons, there is a certain level of instability in

the continuum background and this instability plays a role in limiting the sensitivity of ICP-AES.

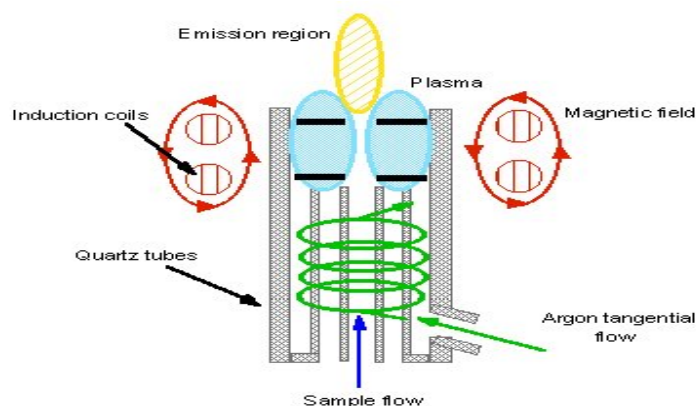


Figure A4-1. ICP Torch

Source: <http://www.chemistry.adelaide.edu.au/external/soc-rel/content/icp.htm>

All ICP-AES systems consist of several components: the sample introduction system, the torch assembly, and the spectrometer. The sample introduction system on the ICP-AES consists of a peristaltic pump, Teflon tubing, a nebulizer, and a spray chamber. The fluid sample is pumped into the nebulizer via the peristaltic pump. The nebulizer generates an aerosol mist and injects humidified Ar gas into the chamber along with the sample. This mist accumulates in the spray chamber, where the largest mist particles settle out as a waste and the finest particles are subsequently swept into the torch assembly. Approximately 1% of the total solution eventually enters the torch as a mist, whereas the remainder is pumped away as waste.

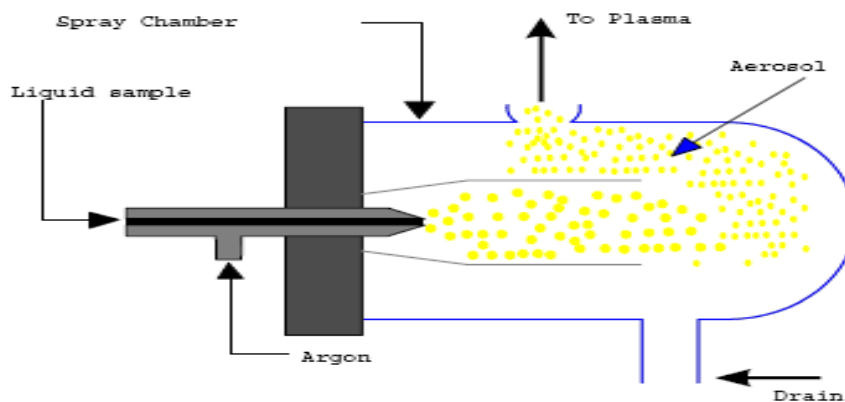


Figure A4-2. Atom formation in ICP nebulizer

Source: <http://www.mrl.ucsb.edu/mrl/centralfacilities/chemistry/icp.pdf>

The fine aerosol mist containing Ar gas and sample is injected vertically up the length of the torch assembly into the plasma. The radio frequency-generated and maintained Ar plasma, portions of which are as hot as 10,000 K, excites the electrons. When the electrons return to ground state at a certain spatial position in the plasma, they emit energy at the specific wavelengths peculiar to the sample's elemental composition.

The plasma is viewed horizontally by an optical channel. Light emitted from the plasma is focused through a lens and passed through an entrance slit into the spectrometer. There are two types of spectrometers used in ICP-AES analysis: sequential (monochromator) and simultaneous (polychromator). The detector (photomultiplier tube) is fixed in space at the far end of the spectrometer. Rotation of the diffraction grating sequentially moves each wavelength into the detector. The computer control ensures that the detector is synchronized with the grating so that the intensity at the detector at any given time is correlated with the wavelength being diffracted by the grating. The operator enters the wavelengths that he or she wishes to detect into the computer, the grating

sequentially moves to the specified wavelengths, and the energy intensity at each wavelength is measured to provide a quantitative result that can be compared to a reference standard. Using standard spectroscopic techniques (e.g., background corrections), sequential ICP-AES can provide extremely flexible and rapid analysis of a number of chemical elements. The spectrometer is flushed with N₂ gas to improve the detection limits of elements with emission wavelengths that are severely compromised by interference with air (e.g., P). This N₂ flush, which is constantly maintained in the instrument regardless of whether such elements are being analyzed, also protects the optics from the corrosive aspects of the atmosphere.

Concentration of extracted metals is determined using an Inductively Coupled Plasma – Atomic Emission Spectrometer (ICP-AES), Thermofischer iCAP 6500 Duo in Precision Analytical Lab, Cleveland, OH. For Hg, cold vapor technique with Perkin Elmer FIMS AS90 mercury analyzer was used. To prepare the instrument for operation, the argon supply is turned on; the torch is started and allowed to warm up for about five minutes. The appropriate wavelength is selected for each element and the line is located for the respective wavelength. The instrument is then calibrated using multi-elements standards.

Samples are introduced through the nebulizer and the measured emission is displayed in the computer. At the end of the analysis, the results are filtered to display only the required parameters. The result is exported to the spread sheet file for conversion of emission readings to concentrations. (ICP References: 20, 25 and 32)

APPENDIX 5

4.1 X-Ray Fluorescence Spectroscopy

When the material is bombarded with X-Ray, it gets excited and release X-Ray fluorescence. This technique is used for the high accurate determination of major elements and broad elemental survey of the sample composition without standards. When high energy photons such as X-Ray or Gamma rays strike any atoms, the atoms may either absorb or scattered the energy. If the atom absorbs energy it is termed as photoelectric effect. The absorption of the energy is higher than that of ionization potential of the atoms; it may results in ionization of the atoms ejecting electrons. The ejection of the electron makes the atom unstable. The electrons are ejected from the inner shell. To fill this gap, other electrons from the higher energy level or higher orbitals fall into the lower shell. While going from higher to lower energy level, energy is released in the form of photon, the magnitude of which is equal to the difference of energy between the two orbitals involved. In this way the material emits energy that is characteristics of the atoms involved. In this process, since the absorption of higher energy results in the release of lower energy, hence the term “fluorescence” is used. In most cases the innermost K and L shell are involved in the process of XRF detection hence X-Ray emission lines are commonly referred to KA, KB, LA or LB (Figure A5-1). K an L refers to the electron shell vacancy being fulfilled and the A and B refer to the source from which the electron originates. Sometimes, as the atom returns to its stable condition, instead of emitting a characteristic x-ray it transfers the excitation energy directly to one of the outer electrons, causing it to be ejected from the atom. The ejected electron is

called an "Auger" electron. This process is a competing process to XRF. Auger electrons are more probable in the low Z elements than in the high Z elements.

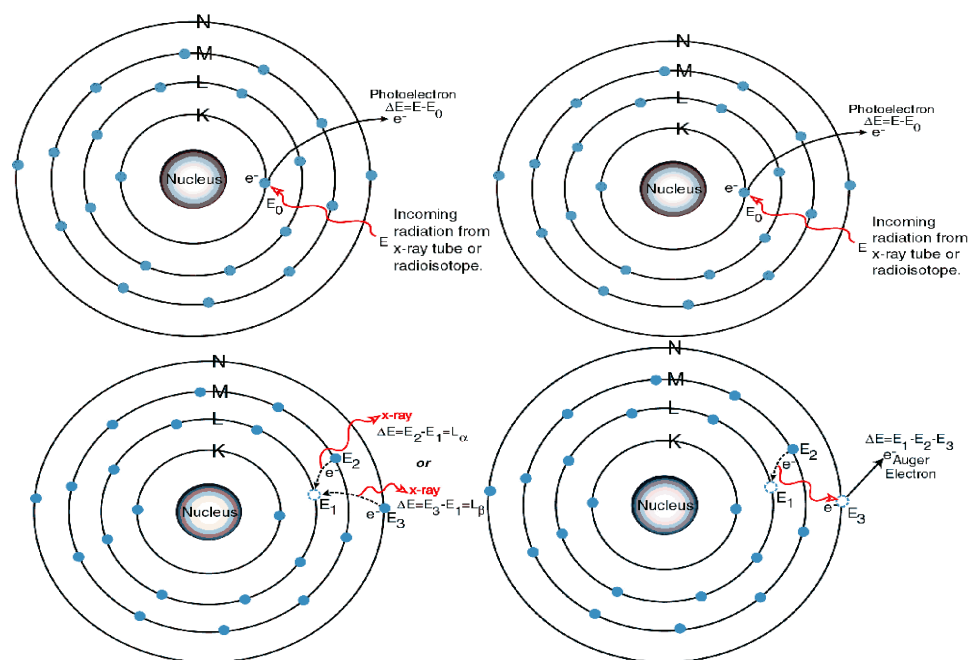


Figure A5-1. XRF Process

Source: <http://www.amptek.com/xrf.html>

There are generally two types of XRF techniques namely, energy dispersive and wavelength dispersive. In wave length dispersive system, a diffraction crystal is used to focus the specific wavelengths onto the detector and in energy dispersive system; the spectrometer focuses all the emitted x-rays onto an energy analyzing detector.

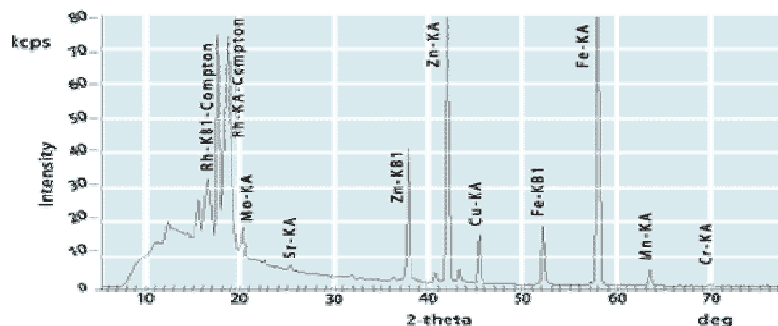


Figure A5-2. Quantitative X-Ray fluorescence scans

Source: <http://www.wcaslab.com/TECH/xrf.htm>

The wavelength dispersive system is more sensitive and has higher resolution. XRF is a non-destructive analytical technique. In principle the lightest elements that can be analyzed is beryllium ($Z=4$) but due to the instrumental limitation it is often difficult to detect elements lighter than sodium ($Z=11$). A background correction and inter-elements correlation is needed.

XRF techniques can be used for both solid and liquid samples such as rock, sediment, coal, plastic, oil, petrol, solutions, blood or wine. Very few to very high concentrations of up to 100% can be analyzed directly without any dilution process. It is simple and fast techniques. It is also one of the most important tools in the analysis of environmental samples such as sludge, sediment and waste.

In the experiment, Bruker axs, S2 ranger machine was used for analysis. The liquid extracts were placed into the double ended (closed with Para-film and cap) sample cup supplied by Premier Lab Supply. The sample was analyzed by water method that has been set-up in the instrument under helium gas by Bruker Corporation. Method was set at run time of 8 minute and 30 minute duration.

Analytical Lines

The spectral lines for the analysis are selected on the basis of intensity, accessibility by the instrument, and lack of line overlaps. Typical lines used, and their wavelengths, are as follows:

Table A5-1: XRF Analytical lines for some elements

Source: http://en.wikipedia.org/wiki/X-ray_fluorescence

Element	Line	wavelength – nm	element	Line	wavelength – nm	Element	Line	wavelength – nm
Li	K α	22.8	Ni	K α 1	0.1658	C	K α	4.47
Be	K α	11.4	Cu	K α 1	0.1541	Pb	L α 1	0.1175
Hg	L α 1	0.1241	Zn	K α 1	0.1435	Ba	L α 1	0.2776
As	K α 1	0.1176	Na	K α 1,2	1.191	Si	K α 1,2	0.7126
Se	K α 1	0.1105	Mg	K α 1,2	0.989	Mo	K α 1	0.07094
Pd	K α 1	0.05859	Al	K α 1,2	0.834	Ag	K α 1	0.05599
V	K α 1	0.2504	Cd	K α 1	0.05357	W	L α 1	0.1476

(XRF References: 15, 16, 17 and 18)